Biofabrication Of Microtissue-Derived Constructs for Articular Cartilage Repair

Gabriela S. Kronemberger[1], Francesca D. Spagnuolo[1], Kyle J. Storey[1], Daniel J. Kelly[1]
[1]Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

Email of Presenting Author: soareskg@tcd.ie

Disclosures: Not applicable

INTRODUCTION

Engineering functional and phenotypically stable articular cartilage is considered one of the greatest challenges in tissue engineering. Modular tissue engineering strategies that use cellular aggregates, microtissues or organoids as building-blocks can potentially be used to fabricate complex hierarchical tissues at scale. The aim of this work was to (i) assess the capacity of cartilage microtissues at different levels of maturation to form scaled-up cartilage grafts in the presence or absence of a temporary supporting hydrogel, and (ii) assess if extrusion based bioprinting of such cartilage microtissues could be used for the biofabrication of functional cartilage grafts.

METHODS

Mesenchymal stem/stromal cell (MSC) derived microtissues were fabricated as described previously (Nulty, Burdis and Kelly, 2021 and Burdis *et al*., 2022). These microtissues were harvested after 2 or 4 days of maturation, manually seeded in agarose wells with or without a supporting oxidized alginate hydrogel and maintained in chondrogenic culture for 6 weeks in either static or dynamic culture conditions. As proof of concept, the microtissues were 3D bioprinted by extrusion-based technique using gelatin and oxidized alginate-based bioinks (Barceló *et al*. 2022).

RESULTS

Over 6 weeks of culture, microtissues that were matured independently for 2 days prior to seeding in oxidized alginate hydrogels generated higher amounts of glycosaminoglycans (GAGs) compared those matured for 4 days. Histological analysis revealed intense staining for GAGs and negative staining for calcium deposits. In contrast, microtissues that were not encapsulated into supporting oxidized alginate stained positive for calcium deposits (Figure 1). These differences in tissue development due to incorporation of a supporting temporary hydrogel were also observed in dynamic culture conditions, where encapsulation in oxidized alginate support a 2-fold increase in sGAG deposition. Furthermore, the microtissues (day 2 maturation level) were 3D bioprinted using gelatin and oxidized alginate based bioinks. It was observed that the microtissues remained viable after the bioprinting process, were able to fuse after 48h, and generated a cartilage tissue that was rich in GAGs and negative for calcium deposits after 6 weeks in culture.

DISCUSSION & CONCLUSIONS

The key conclusions from this study are that: (1) less mature cartilage microtissues (day 2 maturation level) generate superior scaled-up cartilage grafts; (2) encapsulation of microtissues into a supporting oxidized alginate hydrogel supported the development of a less calcified cartilage grafts; (3) microtissues can be successfully used as in extrusion based bioprinting to engineer scaled-up cartilage constructs. This work supports the continued use of cartilage microtissues as biological building blocks in diverse biofabrication platforms.

SIGNIFICANCE/CLINICAL RELEVANCE

In this work, we explored the potential of bone marrow MSC derived microtissues as building-blocks to biofabricate cartilage constructs. A key novelty of our approach is the use of a temporary supporting hydrogel (oxidized alginate) to support the development of a scaled-up graft using numerous cartilage microtissues; such materials will rapidly degrade in culture (Barceló *et al*., 2022), leading to the development of a scaffold-free cartilage tissue that should be less likely to invoke a foreign-body response in *vivo*. The use of 3D bioprinting as a technique to hierarchically fabricate cartilage-like constructs was already explored in the past. However, the final tissues still lack in morpho-functional organization that can impact future *in vivo* outcomes. The use of microtissues as biological units in 3D bioprinting may allow the biofabrication of more organized functional structures leading to better articular cartilage regeneration outcomes.

REFERENCES

Barceló, X.; Eichholz, K.F.; Garcia, O.; Kelly, D.J. Tuning the degradation rate of alginate-based bioinks for bioprinting functional cartilage tissue. Biomedicines 2022, 10, 1621.
Nulty, J., Burdis, R., Kelly, D.J. Biofabrication of Prevascularised Hypertrophic cartilage microtissues for bone tissue engineering. Frontiers in Bioengineering and Biotechnology 2021, 9.
Burdis, R., Chariyev-Prinz, F., Browe, D., Freeman, F., Nulty, J., McDonnell, E., Eichholz, K., Wang, B., Brama, P., Kelly, D. J. Spatial patterning of phenotypically distinct microtissues to engineer osteochondral grafts for biological joint resurfacing. Biomaterials 2022, 289.

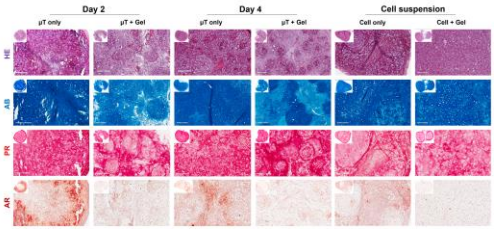

Figure 1: Microtissue-derived constructs show strong staining for sGAG after 6 weeks under static chondrogenic induction. HE: hematoxylin and eosin; AB: alcian blue; PR: picrosirius red; AR: alizarin red. Scale bar: 200μm.